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SHORT COMMUNICATIONS

Blockade of *p*-chloromethamphetamine induced 5-hydroxytryptamine depletion by chlorimipramine, chlorpheniramine and meperidine

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p-CHLOROMETHAMPHETAMINE (CMA, Ro 4/4681) lowers the concentration of both 5-hydroxytryptamine (5HT) and 5-hydroxyindoleacetic acid (5HIAA) in rat brain.[1] The reduction in 5HIAA levels partly results from inhibition of monoamine oxidase,[2] but the cause of the reduction of 5HT remains uncertain.

We have now observed that the reduction in 5HT by CMA can be blocked by several psychoactive drugs.

Male Sprague–Dawley rats (150–250 g) were injected intraperitoneally with CMA (10 mg/kg). Fifteen min later they were injected intraperitoneally with one of the following drugs: chlorimipramine HCl, protriptyline HCl, desmethylimipramine HCl, meperidine HCl, or *d*-chlorpheniramine. Doses in the table refer to the form mentioned here. At various intervals, the rats were decapitated. Their whole brains were removed and analysed for 5HT.[3]

Treatment with chlorimipramine (15 mg/kg) completely blocked the depletion of 5HT for the first 2 hr (Table 1). This protection became less complete for a 4 hr CMA treatment and was nearly ineffective with an 8 hr CMA treatment. The short lasting nature of the blockade by chlorimipramine probably resulted from the slow disappearance of CMA[1] and the rapid metabolism of imipramine-like drugs in rats.[4] When chlorimipramine was given in two 15 mg/kg doses (the second 2 hr after injection of CMA), protection of 5HT from depletion by CMA was complete for 4 hr. Other antidepressives of the imipramine type were less effective: imipramine (25 mg/kg) significantly diminished the depletion of 5HT, while the protection of 5HT by desmethylimipramine was not significant. Protriptyline proved ineffective.

The antihistamine *d*-chlorpheniramine appeared to be at least as potent as chlorimipramine at blocking this effect of CMA on brain 5HT.

A large dose of the addictive-analgesic drug meperidine was also capable of preventing the 5HT depletion induced by CMA. Previous studies in this laboratory show that the drugs tested have no effects on brain 5HT levels in the doses used.

In another experiment, we were able to reverse the CMA induced depletion of 5HT. Rats whose 5HT-levels had been reduced by CMA treatment for 3 hr then received an injection of chlorimipramine. They were sacrificed after an additional 3 hr (see Table 1). Chlorimipramine (15 mg/kg) significantly reversed the depletion of 5HT by CMA. However, when the CMA pretreatment had been 16 hr, a 2-hr treatment with chlorimipramine caused only a slight reversal.

The imipramine like drugs are also capable of blocking the 5HT depletion caused by 4-methyl, α-ethyl metatyramine (H75/12), which seems to displace both 5HT and noradrenaline in the CNS. Carlsson *et al.*[5, 6] concluded that these effects were probably due to blockade of the uptake of H75/12 into the 5HT neurons by the "membrane pump" for amines. It has also been found[7] that chlorpheniramine and meperidine decreased 5HT loss after H75/12. As reported previously,[5–7] some of the drugs studied protect 5HT from depletion more than noradrenaline and vice versa. The order of potency in protection of 5HT from depletion by H 75/12 was chlorimipramine and chlorpheniramine > imipramine > protriptyline > meperidine > desmethylimipramine. In the present study with CMA, the order of potency was generally similar: chlorimipramine and chlorpheniramine > imipramine > meperidine and desmethylimipramine > protriptyline.

A possible explanation for the action of CMA is that it utilises the membrane pump for amines in the 5HT neurons and consequently that the accumulation of CMA in 5HT neurons is prevented by chlorimipramine, chlorpheniramine and meperidine. However, one must consider the possibility that a metabolite of CMA is partly responsible for the 5HT loss, and that formation of this metabolite is prevented by the drugs tested.[8] It seems unlikely that formation of this hypothetical CMA metabolite should be most strongly blocked by precisely those imipramine like drugs which most strongly potentiate endogenous 5HT *in vivo*[9] and which most strongly inhibit neuronal uptake of 5HT *in vitro* and *in vivo*.[10, 11]

TABLE 1. BLOCKADE OF THE 5HT LOSS INDUCED BY *p*-CHLOROMETHAMPHETAMINE (CMA)

Drug (mg/kg)	CMA treatment (hr)	Brain 5HT (% of control)	
		CMA	CMA + DRUG
Chlorimipramine (15)	2	71 ± 3	104 ± 13*
Chlorimipramine (2 × 15)	4	34 ± 10	103 ± 10†
Chlorimipramine 15	4		82 ± 10†
Chlorimipramine 5	4		43 ± 10
Chlorimipramine 15	4		38 ± 10
Chlorimipramine (15)	8	32 ± 4	45 ± 3*
Imipramine (25)	3	35 ± 11	73 ± 11*
Protriptyline (25)	3		39 ± 11
Desmethylimipramine (25)	3		65 ± 11
d-Chlorpheniramine (25)	3		113 ± 11†
Meperidine (50)	3		89 ± 11†
Chlorimipramine (15) (3 hr before death)	6	41 ± 5	91 ± 18*
Chlorimipramine (15) (2 hr before death)	18	50 ± 9	64 ± 6

* $P < 0.05$.
† $P < 0.01$.

All rats were treated with *p*-chloromethamphetamine (CMA). Some were injected with a second drug 15 min later, or as in the last two experiments, 3 or 2 hr before sacrifice. Each value is the mean ± S.E.M. of four determinations. The concentration of 5HT in normal controls was 0·37 ± 0·02 μg/g. Significance of difference between CMA and CMA plus drug groups was calculated by analysis of variance or simple Student's *t*-test.

The above findings suggest that in order to act CMA requires concentration by continuous uptake into the serotonergic neurons. If uptake is blocked, for example with chlorimipramine, the highly lipid soluble CMA can escape. The actual reduction in 5HT concentration might result from a displacement from the granules or a reserpine like effect with subsequent loss of 5HT from the brain.[12] It is also possible that CMA causes an initially reversible inhibition of tryptophan hydroxylase.[13] The former possibility is supported by histochemical evidence (to be published) that CMA can release 5HT into the extraneuronal space in nialamide treated rats.

However, another histochemical study supports both possibilities. CMA reduced the 5HT fluorescence in both cranial and caudal halves of transected rat spinal cords, but there was a smaller reduction in the caudal half. This effect is therefore unlike that of a tryptophan hydroxylase inhibitor, which causes practically no depletion of 5HT caudal to a transection.[14] The effect is also different from reserpine like agents[14] and displacing agents[15] which reduce 5HT fluorescence equally in both halves. Thus, a combination of effects is likely to be responsible for the 5HT depletion by CMA.

Our results may prove helpful in discovering other drugs which can block the 5HT "membrane pump". CMA has some advantages over H75/12 as a test agent: the former is a long-acting, potent depletor, while the latter requires repeated high doses.

It should be realised that CMA not only lowers 5HT levels, but also may directly stimulate 5HT receptors* and has central amphetamine like effects.[16] Therefore CMA should not be used as a selective drug for revealing the functional effects of decreased 5HT neurotransmission.

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The action of some ω-diazoacetophenones on purine biosynthesis, as compared with the action of L-azaserine

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THE SUBSTITUTED L-serine, *O*-diazoacetyl-L-serine (L-azaserine),[1] has been shown to be similar in structure to L-glutamine,[2] and to interfere in the biosynthesis of the purines by competing with L-glutamine in the amination of formyl-glycinamide ribotide (FGAR) to formyl-glycinamidine ribotide (FGAM).[3,4] In spite of the high degree of specificity and potency shown by L-azaserine in *in vitro* tests, clinical studies of L-azaserine against neoplastic conditions showed it to have little beneficial effect.[5,6] It appeared that L-azaserine was extensively inactivated *in vivo*. We thought it possible that analogous ω-diazoacetophenones (Fig. 1) might retain the specific enzyme blocking action of L-azaserine, whilst being more stable to metabolic breakdown, and further if degraded, the breakdown products might have antineoplastic activity.